A minimal model for translational control of protein expression

Arvind R. Subramaniam[1] and Erin K. O'Shea[1, 2]

It is now possible to routinely monitor translation with single-nucleotide resolution. However, our ability to formulate a predictive model of protein synthesis from this data is still limited. By combining experimental and modeling strategies at the genome scale, we identified molecular events that determine the ribosome density on mRNAs and the expression level of proteins during starvation for single amino acids in *Escherichia coli*. Notably, we delineated the quantitative contribution of ribosome traffic jams and aborted translation to the overall rate of protein synthesis. Our integrated approach reveals a novel set of ingredients for a mechanistically-accurate, computational model of translation.

BACKGROUND

PROTEIN synthesis involves initiation of translation by ribosomes on mRNA templates, which is followed by a series of elongation steps during which amino acids are added to the growing polypeptide chain. Initiation is the rate-limiting step of protein synthesis during nutrient-rich growth of cells. However, during perturbations such as nutrient starvation or overproduction of proteins, elongation rate of ribosomes can decrease significantly. Computational models of translation aim to predict the effect of changes in elongation rate on the overall rate of protein synthesis.

Most computational models of translation are variants of the totally asymmetric simple exclusion process (TASEP), a paradigmatic model in non-equilibrium physics *(1)*. These models postulate that a decrease in ribosome elongation rate on an mRNA causes a traffic jam of trailing ribosomes, which then attenuates the rate of protein synthesis. However, the relevance of ribosome traffic jams to measured *in vivo* rates of protein synthesis remains to be characterized.

We recently found that starvation for single amino acids in the bacterium *E. coli* caused over a 100-fold decrease in protein synthesis rate, that was caused by the presence of certain starvation-sensitive codons in the mRNA *(2)*. This starvation-specific effect of codons determined the growth response of *E. coli* subjected to amino acid downshift, and the biofilm-forming ability of the bacterium *Bacillus subtilis (3)*. To determine the mechanistic basis for this large effect of synonymous codons, we measured the genome-wide ribosome density on messenger RNAs in *E. coli* during starvation for single amino acids using the ribosome profiling method *(4)*. We then used this measurement to rigorously constrain a whole-cell model of translation *(5)*.

[1]FAS Center for Systems Biology, and Dept. of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138

[2]Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138

Email: asubram@fas.harvard.edu , erin_oshea@harvard.edu

Funding: NIH 1K99GM107113 (A.R.S), Howard Hughes Medical Institute (E.K.O.)

RESULTS

Starvation for single amino acids led to ribosome pausing, but only at a subset of codons cognate to the limiting amino acid. Quantitative prediction of this codon specificity required accounting for the differential kinetics of aminoacylation among tRNA isoacceptors in addition to a supply-demand balance *(6)*.

Intragenic ribosome density was skewed towards the 5' end of mRNAs. Pausing of a single ribosome caused a traffic jam of trailing ribosomes. In agreement with a TASEP model of translation, the severity of ribosome traffic jams was determined both by the intragenic location of the ribosome pause site and the translation efficiency of the mRNA. However the length of ribosome traffic jams was incommensurate with the quantitative effect of ribosome pause sites on protein synthesis rate.

Incorporating translation abortion at ribosome pause sites into the TASEP model was sufficient to predict the effect of ribosome pausing on measured ribosome density and protein synthesis rate. Further, evidence indicated that the transfer-messenger RNA (tmRNA/ssrA) is the primary effector of translation abortion at ribosome pause sites during amino acid starvation.

Our generalized model of translation, formulated in the specific context of amino acid starvation of *E. coli*, also accounted for the molecular events during artificial overproduction of proteins and during amino acid starvation in eukaryotes.

CONCLUSIONS

Our work provides an experimentally-validated set of ingredients for the systematic modeling of protein synthesis. More generally, it highlights the ability of deep-sequencing approaches to rigorously constrain computational models.

REFERENCES

1. C. T. MacDonald, J. H. Gibbs, Concerning the kinetics of polypeptide synthesis on polyribosomes, Biopolymers 7, 707–725 (1969).

2. A. R. Subramaniam, T. Pan, P. Cluzel, Environmental perturbations lift the degeneracy of the genetic code to regulate protein levels in bacteria, Proc. Natl. Acad. Sci. 110, 2419–2424 (2013).

3. A. R. Subramaniam et al., A serine sensor for multicellularity in a bacterium, eLife 2 (2013).

4. N. T. Ingolia, S. Ghaemmaghami, J. R. S. Newman, J. S. Weissman, Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling, Science 324, 218–223 (2009).

5. P. Shah, Y. Ding, M. Niemczyk, G. Kudla, J. B. Plotkin, Rate-limiting steps in yeast protein translation, Cell 153, 1589–1601 (2013).

6. J. Elf, D. Nilsson, T. Tenson, M. Ehrenberg, Selective charging of tRNA isoacceptors explains patterns of codon usage, Science 300, 1718–1722 (2003).